Abstract

The response rates of HER1 antibody monotherapy (<1%) is comparable to the added response rate of HER1/HER2 therapy when used in combination with chemotherapy (<1%) in patients with recurrent and/or metastatic squamous cell carcinomas of the head and neck (SCCHN), whereas all HER1/HER2-targeting agents (HER1 and/or gene-coproduction number by fluorescent in-situ hybridization (FISH) do not correlate with response. Therefore, alternative or proven protein expression and/or subcellular protein expression patterns were as also evaluated in patients to identify patients most likely to benefit from HER1/HER2 therapy. In vitro, we evaluated expression of HER1, HER2, HER3, cMET, and HER4 protein expression in tumor cell lines using the fluorescent in-situ hybridization (FISH) technique. The correlation of HER1/HER2 with HER1/HER2 was significant for 100% of SCCHN compared to other carcinomas of the head and neck. In this study, we analyzed the expression of HER1, x, and cMET using the VeraTag RPA kit; this test is the only test currently available for the detection of HER1/HER2 expression in SCCHN tumors. Our results identified two distinct protein expression profiles: an activated HER1 profile at the profile characterized by the HER1/HER2 expression levels of HER1, HER2, HER3, and cMET, and at the level of HER1/HER2. We found that the HER1/HER2 correlated with sensitivity to HER1/HER2-targeted therapies and that the profile correlated with with either sensitivity or resistance to HER1/HER2-targeted therapies. HER1 protein levels measured by VeraTag assay varied over a 16-fold dynamic range and significantly correlated with HER1 IHC staining and mRNA levels determined by qPCR. HER2 and HER3 protein expression varied over a ~25-to-1 dynamic range and correlated with mRNA levels by qPCR. The HER1 expression of all three subcellular protein expression patterns (HER1/HER2, HER1pY1173, and HER1pPan) were highly activated, and thus it was activated in all HER1/HER2 targets. The HER1/HER2 expression summarized in Table 1 and Figure 1. Fututives were designed to further validate the HER1 IHC activation as a predictor of response to HER1-targeted therapies and/or the alteration of response due to HER1/HER2 or a cMET expression.

The expression of HER1, HER2, HER3, and cMET protein expression patterns were significantly higher in immunopositive tumors compared to tumors negative for expression. Future studies were designed to further validate the HER1/HER2 activation as a predictor of response to HER1-targeted therapies and/or the alteration of response due to HER1/HER2 or a cMET expression.

1. Head and Neck Tumors

The squamous cell carcinomas of the head and neck tumors used for the study were separated into two groups, S-8 and S-15, which were analyzed by two separate sets of assays testing and were both normative to allow direct comparison of all tumors.

2. VeraTag Assays and Performance

Control cell lines were assessed together with the tumors. The distribution of the assay signal (black spot) is shown together with the output controls. However, for tumors containing tumors and control cell lines, tumor samples were macro-dissected to 1% tumor and 99% non-tumor elements.

3. Correlations with IHC, FISH, and mRNA

VeraTag assays for the head and neck squamous cell carcinomas were composed of (HER1, HER2, HER3, cMET, and HER4) and (HER1, HER2, HER3, cMET, and HER4) from the same tumor. Significant correlations (p < 0.05) were found for all HER1-based comparisons. HER1/HER2 VeraTag correlates with mRNA with p2 > 0.35 and p2 = 0.35, respectively.

4. HER1 Activation in SCCHN

Three different analyses of the protein expression profiles for the SCCHN tumors obtained from the VeraTag system (a) were tested for HER1 IHC. (b) were tested for HER1/HER2 using the HER1/HER2 expression and (c) were tested for HER1/HER2 expression. Three laboratories were used to test for HER1/HER2 expression. The HER1 expression is similar in magnitude to the response rate seen with HER1-targeted therapies. Future studies are designed to further validate the HER1 activation as a predictor of response to HER1-targeted therapies and/or the alteration of response due to HER1/HER2 or a cMET expression.

5. VeraTag Correlations in SCCHN

The activated HER1 IHC tumors have a large distribution in all HER1-based measurements, the HER1 expression profile was highly correlated with HER1 mRNA expression. The HER1 expression was highly correlated with HER1 mRNA expression. The majority of tumors exhibit a H1T HER1 expression pattern. H1T HER1 expression was highly activated in the majority of tumors exhibiting high mRNA levels for HER1/HER2.

6. Activated HER1 expression vs HER1

The activated HER1 IHC tumors have a large distribution in all HER1-based measurements, the HER1 expression profile was highly correlated with HER1 mRNA expression. The HER1 expression was highly correlated with HER1 mRNA expression. The majority of tumors exhibit a H1T HER1 expression pattern. H1T HER1 expression was highly activated in the majority of tumors exhibiting high mRNA levels for HER1/HER2.

Summary

- Quantitative FFPE assays were developed using the VeraTag technology to measure protein levels of the HER1 family and c-MET receptor tyrosine kinases in head and neck squamous cell carcinomas.
- Using macro-dissected samples, a range of HER1 protein levels were measured by VeraTag assays in ~25% of the SCCHN tumors, whereas a similar range of HER1 expression in the biopsies was determined by HER1 mRNA expression in the biopsies. HER1 expression was measured by FISH or qPCR.
- The HER1/HER2 total expression measurements were significantly different for HER1 IHC and mRNA levels determined by qPCR. HER1 IHC gene copy number is significantly correlated with the HER1 expression levels in the majority of tumors exhibiting high HER1 expression. HER1 mRNA levels were significantly different for HER1 IHC and mRNA levels, whereas HER1 expression was significantly correlated with the HER1 expression levels in the majority of tumors exhibiting high mRNA levels for HER1/HER2.
- Summary of HER1/HER2 expression and activation.

- HER1/HER2 expression and activation measurements were significantly different for HER1 IHC and mRNA levels determined by qPCR. HER1 IHC gene copy number is significantly correlated with the HER1 expression levels in the majority of tumors exhibiting high HER1 expression. HER1 mRNA levels were significantly different for HER1 IHC and mRNA levels, whereas HER1 expression was significantly correlated with the HER1 expression levels in the majority of tumors exhibiting high mRNA levels for HER1/HER2.

- HER1 expression measurements were significantly different for HER1 IHC and mRNA levels determined by qPCR. HER1 IHC gene copy number is significantly correlated with the HER1 expression levels in the majority of tumors exhibiting high HER1 expression. HER1 mRNA levels were significantly different for HER1 IHC and mRNA levels, whereas HER1 expression was significantly correlated with the HER1 expression levels in the majority of tumors exhibiting high mRNA levels for HER1/HER2.

- Tumors with the activated HER1 signature do not strictly as a single group with standard metabolic or HER1/HER2 IHC expression. The HER1 expression was highly correlated with HER1 mRNA expression. HER1 mRNA levels were significantly different for HER1 IHC and HER1 protein levels determined by qPCR.

- HER1 expression and activation measurements were significantly different for HER1 IHC and mRNA levels determined by qPCR. HER1 IHC gene copy number is significantly correlated with the HER1 expression levels in the majority of tumors exhibiting high HER1 expression. HER1 mRNA levels were significantly different for HER1 IHC and mRNA levels, whereas HER1 expression was significantly correlated with the HER1 expression levels in the majority of tumors exhibiting high mRNA levels for HER1/HER2.